Biochemical Pharmacology, Vol. 36, No. 22, pp. 3981–3985, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

EFFECTS OF ETHANOL, ACETALDEHYDE AND DISULFIRAM ON THE METABOLISM OF BIOGENIC ALDEHYDES IN ISOLATED HUMAN BLOOD CELLS AND PLATELETS

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(*Received* 3 *February* 1987; *accepted* 9 *June* 1987)

Abstract—The effects of ethanol, acetaldehyde and disulfiram on the metabolism of biogenic aldehydes were measured in different human blood fractions. Intact erythrocytes, leukocytes and platelets were incubated in phosphate-buffered saline with 3,4-dihydroxyphenylacetaldehyde (DOPAL) or 5-hydroxyindole-3-acetaldehyde (5-HIAL), the aldehydes derived from dopamine and serotonin, respectively. The disappearance of the aldehyde and the formation of acid and alcohol metabolites were analysed in the presence of different concentrations of ethanol, acetaldehyde or disulfiram using high-performance liquid chromatography with electrochemical detection. Ethanol at a concentration of 20 mM did not affect the biogenic aldehyde metabolism. High concentrations of acetaldehyde caused a dose-dependent inhibition of the disappearance rate of the biogenic aldehydes and of the formation rate of acid metabolites. In incubations with leukocytes or platelets, the inhibition of the acid formation was associated with a slight increase in the formation of the alcohol metabolites. Disulfiram at a concentration of 50 μM totally inhibited the metabolism of DOPAL and 5-HIAL in incubations with erythrocytes or platelets, whereas much less inhibition was observed in incubations with leukocytes.

Biogenic aldehydes are formed by oxidative deamination of biogenic amines by the action of monoamine oxidase (MAO; EC 1.4.3.4), which is present in most tissues. The aldehydes are further metabolized either to the corresponding acids by aldehyde dehydrogenase (ALDH; EC 1.2.1.3) or to the corresponding alcohols by aldehyde reductase (ALR; EC 1.1.1.2) or alcohol dehydrogenase (ADH; EC 1.1.1.1).

It was recently shown that biogenic aldehydes are metabolized by human erythrocytes, leukocytes and platelets [1]. When the aldehydes derived from dopamine or serotonin (5-hydroxytryptamine) were incubated with intact erythrocytes, only the acid metabolites were formed. This indicated that the aldehydes were metabolized by ALDH, which is present in human erythrocytes [2]. However, when these aldehydes were incubated with leukocytes or platelets, both the acid and alcohol metabolites were formed, indicating the presence of both ALDH and ALR/ADH enzymes [1]. No metabolism of these aldehydes was observed in plasma.

The concentration of biogenic aldehydes has been suggested to increase in the CNS and peripheral systems during ethanol metabolism, due to competitive inhibition of ALDH by acetaldehyde [3]. Furthermore, a shift in the biogenic aldehyde metabolism, from oxidative to reductive pathways, has been observed during ethanol metabolism [4, 5]. This shift may be due to inhibition of the oxidative pathway by acetaldehyde. However, it has also been proposed to be due to an alteration in the NAD/NADH ratio or to a facilitated binding of biogenic aldehydes to the ADH–NADH complex, which exists during ethanol metabolism [6]. The biogenic aldehyde level might also be altered by direct effects of ethanol, and other drugs, on the turnover of catecholamines [7]. Finally, drugs such as disulfiram (Antabuse) and calcium carbimide (Temposil, Dipsan), used in the treatment of alcoholism, affect the level by direct inhibition of ALDH, and, in combination with ethanol, also by competitive inhibition of ALDH by acetaldehyde.

This study was carried out to examine the effects of ethanol, acetaldehyde and disulfiram on the metabolism of 3,4-dihydroxyphenylacetaldehyde and 5-hydroxyindole-3-acetaldehyde, the aldehydes derived from dopamine and serotonin, respectively, in isolated intact human erythrocytes, leukocytes and platelets.

MATERIALS AND METHODS

Chemicals. NAD, NADH, dopamine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-hydroxytryptamine creatinine sulphate complex, 5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), 5-hydroxytryptophol (5-HTOH) and sodium bisulphite were obtained from Sigma Chemical Co. (St. Louis, MO). 3,4-Dihydroxyphenylethanol (DOPET) was obtained from Serva AG (Heidelberg, F.R.G.). EDTA, sodium octylsulphate and acetaldehyde were obtained from Merck AG (Darmstadt, F.R.G.) and

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the acetaldehyde was freshly distilled before use. Disulfiram (Fluka AG, Buchs, Switzerland) was recrystallized twice from 99.5% ethanol. The bisulphite forms of 3,4-dihydroxyphenylacetaldehyde (DOPAL) and 5-hydroxyindole-3-acetaldehyde (5-HIAL) were prepared enzymatically by the use of rat liver MAO as described by Pettersson and Tottmar [8]. The free aldehydes were prepared from the bisulphite forms by ether extraction [9]. The concentrations of DOPAL and 5-HIAL, and of the acid and alcohol standards, were determined as described by Helander and Tottmar [10].

Fractionation of blood. Fresh human citrated blood was obtained from the University Hospital of Uppsala, Sweden. Erythrocytes, leukocytes and platelets were isolated by Percoll density gradient centrifugation technique (Pharmacia AB, Uppsala, Sweden) as previously described by Helander and Tottmar [1]. The blood cells and platelets were counted on a Hycel HC 333 cell counter (Clinicon, Mannheim, F.R.G.) equipped with a multi-channel analyser.

Assay conditions. Assays were performed by following the disappearance of the aldehyde and the formation of the acid and alcohol metabolites when DOPAL or 5-HIAL were incubated with erythrocytes, leukocytes or platelets in the presence of different concentrations of ethanol, acetaldehyde or disulfiram. Erythrocytes or leukocytes ($5 \cdot 10^7$ cells/ml) or platelets ($5 \cdot 10^8$ platelets/ml) were suspended in 5 ml of phosphate-buffered saline (PBS, 10 mM NaH_2PO_4 and 10 mM Na_2HPO_4 in 0.15 M NaCl), pH 7.4, containing 50 μM EDTA, and were incubated under gentle shaking in sealed polyethylene flasks at 37° in a water bath. The assay mixtures were preincubated for 5 min before addition of the substrate. In assays studying the effects of disulfiram, the disulfiram was added at the start of the preincubation and the inhibition was measured after 15 and 60 min of incubation.

The assays were started by addition of the biogenic aldehyde together with ethanol or acetaldehyde. Aliquots of 0.5 ml were taken at different times during 60 min of incubation and were immediately deproteinized by mixing with 0.125 ml ice-cold 16.8% (w/v) perchloric acid (PCA). The samples were centrifuged at 20,000 *g* for 10 min and the supernatants were immediately analysed or stored at −80° until analysis. The metabolites were stable for at least one week in samples stored at −80°.

Analysis of metabolites with HPLC. The aldehydes and the acid and alcohol metabolites were analysed using high-performance liquid chromatography (HPLC) with electrochemical detection. The chromatographic system used was composed of a Milton Roy Minipump, Mod. 396 (Laboratory Data Control, Riviera Beach, FL), and a Rheodyne injector, Mod. 7125, with a 20 μl sample loop (Rheodyne Inc., Cotati, CA). An electrochemical detector, Mod. LC-3, from Bioanalytical Systems Inc. (West Lafayette, IN) with a glassy carbon working electrode was used, and the potential was set to +0.75 V versus an Ag/AgCl reference electrode. The analytical column was a Nucleosil C_{18} reversed phase column (250 × 4.0 mm i.d., 5 μm particle size) from Macherey-Nagel (Düren, F.R.G.).

The mobile phase was pumped at a flow rate of 1 ml/min and consisted of 105 mM citrate, 2.5% (v/v) methanol, 0.002% (w/v) EDTA and 5 mg/l of sodium octylsulphate, pH 2.2. The mobile phase was filtered under vacuum through a 0.45 μm Millipore HA filter and deaerated before use. Chromatography was carried out at room temperature.

This system allowed simultaneous determination of DOPAL and 5-HIAL, the acid (DOPAC and 5-HIAA) and alcohol (DOPET and 5-HTOH) metabolites, and also the parent amines, DA and 5-HT, respectively [1]. Standard solutions (0.1–1.0 μM) were prepared in 3.4% (w/v) PCA and the amounts of the metabolites were calculated from the peak-heights in the chromatograms. Good linearity between peak-heights and amounts of metabolites was observed in the concentration range used.

RESULTS

Effects of ethanol on the metabolism of biogenic aldehydes

Ethanol at a concentration of 20 mM did not affect the biogenic aldehyde metabolism, when 1 μM DOPAL or 5-HIAL were incubated with erythrocytes, leukocytes or platelets. Not even when erythrocytes were incubated with 20 mM ethanol for 2 hr before the start of the assay were any effects on the aldehyde metabolism observed.

Effects of acetaldehyde on the metabolism of biogenic aldehydes

When erythrocytes, leukocytes or platelets were incubated with 1 μM DOPAL or 5-HIAL in the presence of different concentrations of acetaldehyde, a dose-dependent inhibition of the disappearance rate of the biogenic aldehydes and of the formation rate of acid metabolites were observed. However, the effects on the biogenic aldehyde disappearance were less pronounced than were the effects on the acid formation.

In incubations with erythrocytes and 1 μM DOPAL, approximately 45 and 75% inhibition of the DOPAC formation were observed after 5 min at acetaldehyde concentrations of 100 and 500 μM, respectively (Fig. 1a). The corresponding inhibition of the DOPAL disappearance was about 35 and 65%. The final recovery of the aldehyde as the acid metabolite (when the reaction was completed) was significantly ($P < 0.05$; Wilcoxon two-tailed rank sum test) decreased in incubations in the presence of 500 μM acetaldehyde, as compared to the value in the absence of acetaldehyde. The corresponding results obtained in incubations with 5-HIAL as the substrate were more pronounced, and incubations with both 100 and 500 μM acetaldehyde resulted in a significant ($P < 0.01$) decrease in the final recovery of 5-HIAA (Fig. 1b).

In incubations with leukocytes and 1 μM DOPAL, about 50 and 70% inhibition of the DOPAC formation were observed after 5 min in the presence of 100 and 500 μM acetaldehyde, respectively (Fig. 2). The decrease in acid formation was associated with a slight increase in the formation of the alcohol metabolite. The disappearance of DOPAL was

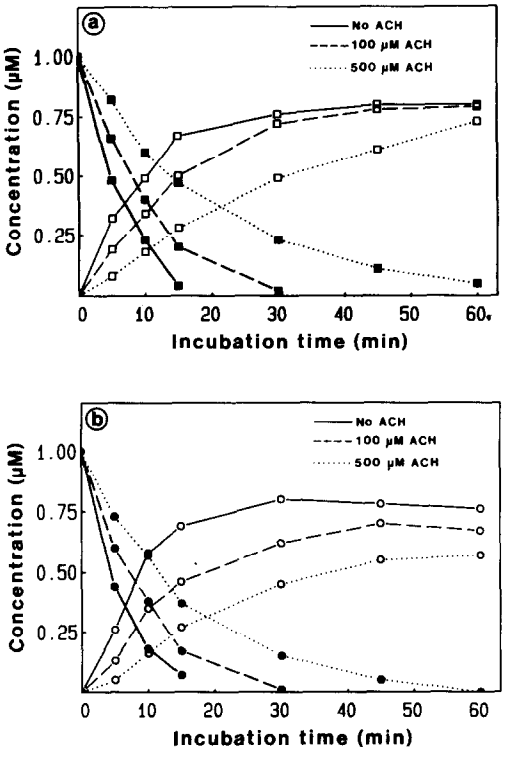

Fig. 1. Effects of different concentrations of acetaldehyde (ACH) on the metabolism of DOPAL and 5-HIAL in isolated human erythrocytes. Intact erythrocytes were suspended in PBS, pH 7.4 ($5 \cdot 10^7$ cells/ml), and were incubated at 37° with 1 μM of the aldehydes. (a) The disappearance of DOPAL (■) and the formation of DOPAC (□) and (b) the disappearance of 5-HIAL (●) and the formation of 5-HIAA (○) were measured in the absence of acetaldehyde and in the presence of 100 or 500 μM acetaldehyde. Aliquots were taken at different times for HPLC analysis of metabolites. The results are the means from duplicate determinations on five different erythrocyte preparations.

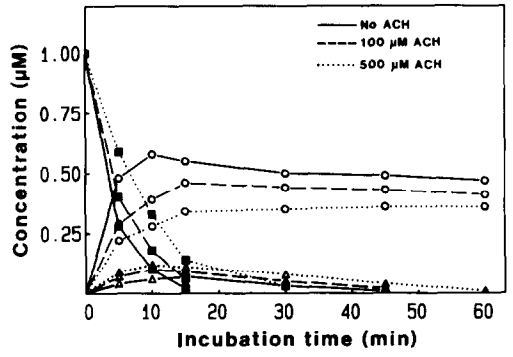

Fig. 2. Effects of different concentrations of acetaldehyde (ACH) on the metabolism of DOPAL in isolated human leukocytes. Intact leukocytes were suspended in PBS, pH 7.4 ($5 \cdot 10^7$ cells/ml), and were incubated at 37° with 1 μM DOPAL. The disappearance of DOPAL (■) and the formation of DOPAC (○) and DOPET (△) were measured in the absence of acetaldehyde and in the presence of 100 or 500 μM acetaldehyde. Aliquots were taken at different times for HPLC analysis of metabolites. The results are the means from duplicate determinations on five different leukocyte preparations.

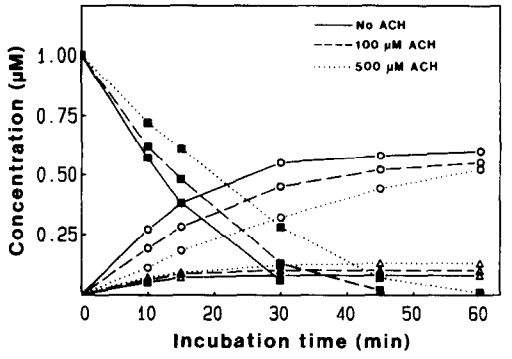

Fig. 3. Effects of different concentrations of acetaldehyde (ACH) on the metabolism of DOPAL in isolated human platelets. Intact platelets were suspended in PBS, pH 7.4 ($5 \cdot 10^8$ platelets/ml), and were incubated at 37° with 1 μM DOPAL. The disappearance of DOPAL (■) and the formation of DOPAC (○) and DOPET (△) were measured in the absence of acetaldehyde and in the presence of 100 or 500 μM acetaldehyde. Aliquots were taken at different times for HPLC analysis of metabolites. The results are the means from duplicate determinations on five different platelet preparations.

inhibited by about 20 and 45%, respectively. The final recovery of DOPAL as DOPAC was significantly ($P < 0.05$) decreased, the formation of DOPET significantly ($P < 0.01$) increased, and the total recovery of DOPAL as DOPAC and DOPET significantly decreased ($P < 0.05$) in incubations with 500 μM acetaldehyde (Fig. 2). Similar results were obtained in incubations with 5-HIAL as the substrate.

In incubations with platelets and 1 μM DOPAL, about 45 and 75% inhibition of the DOPAC formation were observed after 5 min in the presence of 100 and 500 μM of acetaldehyde, respectively (Fig. 3). As observed in incubations with leukocytes, the decrease in acid formation was associated with a slight increase in the formation of the alcohol metabolite. The disappearance of DOPAL was inhibited by about 25 and 45%, respectively. The final recovery of DOPAL as DOPAC was significantly ($P < 0.01$) decreased and the formation of DOPET significantly ($P < 0.01$) increased in incubations with 500 μM acetaldehyde (Fig. 3). However, the total recovery of DOPAL as DOPAC and DOPET was not significantly decreased. Similar results were obtained in incubations with 5-HIAL as the substrate.

Effects of disulfiram on the metabolism of biogenic aldehydes

When erythrocytes were incubated with 1 μM of DOPAL or 5-HIAL in the presence of 50 μM disulfiram, the metabolism (i.e. acid formation) was almost completely inhibited. In incubations with 1 μM disulfiram, an 85% inhibition of the acid formation was observed. A slight disappearance of the aldehydes were observed during the incubations. When platelets were incubated in the presence of 50 μM disulfiram, both the formation of acid and alcohol metabolites were totally inhibited. However, when incubations were performed with leukocytes

and 50 μM disulfiram, only a 25% inhibition of the acid formation was observed after 15 min, and no decrease at all could be observed after 60 min of incubation, as compared to the normal values.

DISCUSSION

In both brain and peripheral systems of humans, DOPAL and 5-HIAL are predominantly metabolized by the oxidative pathway (by ALDH), whereas the aldehyde of epinephrine (E) and norepinephrine (NE) is preferentially reduced in the brain (by ALR or ADH) but oxidized in peripheral systems [11, 12]. However, the relative proportion of the reduced metabolites formed has been shown to increase during ethanol oxidation [4, 5]. This shift was shown to be due to competitive inhibition of ALDH by acetaldehyde and not, which has been suggested, to a change in the NAD/NADH ratio, caused by the oxidation of ethanol and acetaldehyde by the NAD-dependent enzymes ADH and ALDH [13].

In the present study on human blood fractions, assays were performed with the aldehydes of DA and 5-HT. No assays were performed with the aldehyde of E and NE, since the recovery of the bisulphite-free aldehyde was very low after the ether extraction.

No effects on the metabolism of DOPAL and 5-HIAL were observed after incubation of erythrocytes with 20 mM ethanol for 2 hr before the start of the assay. It has previously been reported that acute administration of ethanol to nonalcoholic subjects, as well as incubation of erythrocytes with about 20 mM ethanol for 2 hr, resulted in an increase in the erythrocyte ALDH activity [14]. No such effects were, however, observed in a similar study on patients undergoing alcohol detoxification [15].

In the present study, acetaldehyde caused a dose-dependent inhibition of the biogenic aldehyde metabolism and, in incubations with leukocytes or platelets, also a slight shift in the metabolism from oxidative to reductive pathways. Rather high concentrations of acetaldehyde, as compared to those of the biogenic aldehydes, were, however, needed to obtain a significant inhibition. These results are consistent with a recent study on partially purified human blood ALDH [16], where the K_m value for acetaldehyde was shown to be 180 times higher than for the biogenic aldehyde DOPAL. Since acetaldehyde acts as a competitive inhibitor, a similar excess of acetaldehyde is thus needed in order to obtain a 50% inhibition of the biogenic aldehyde metabolism (if the assays are performed at substrate saturation levels). The determination of the K_m values was, however, performed at different pH and temperature and also in a different buffer, and it is thus possible that the relation between the K_m values is somewhat different at the conditions used in the present study. In a recent study on human liver cytosolic ALDH II (pI about 5.3), which is similar to the erythrocyte ALDH [16, 17], performed at pH 7 and 25°, the K_m value for acetaldehyde was found to be 20 and 120 times higher than for 5-HIAL and DOPAL, respectively [18].

The ALDH activity in human whole blood is almost exclusively due to the activity of the erythrocytes [16]. Human blood ALDH (i.e. erythrocyte ALDH) is very sensitive to disulfiram [16, 17]. In this and a previous study [1], the ALDH activity in erythrocytes and platelets was totally inhibited by disulfiram, whereas the leukocyte ALDH was much less affected. These results indicate that a different ALDH isozyme is present in leukocytes as compared to erythrocytes and platelets, and that the leukocyte ALDH might be similar to the liver mitochondrial ALDH I (pI about 4.9), which is less sensitive to disulfiram [19]. However, ALDH I has similar affinity for acetaldehyde and biogenic aldehydes as the substrates [18], and one would then expect a more pronounced inhibition of the biogenic aldehyde metabolism by acetaldehyde in incubations with leukocytes than in incubations with erythrocytes or platelets. Since the results obtained with leukocytes were similar to those obtained with erythrocytes or platelets, this indicates that the ALDH activity in human leukocytes is not caused solely by an isozyme similar to ALDH I. Further characterization of the human leukocyte ALDH activity is needed for clarification of these results.

In incubations with platelets, both the oxidative and the reductive pathways were inhibited by disulfiram. This suggests that the ALDH activity in platelets is similar to ALDH II. Furthermore, the results show that the aldehyde reducing activity in human platelets is sensitive to disulfiram.

The biogenic aldehydes were previously considered as inactive intermediates, since they lacked the biological activities of the parent amines [20]. However, biogenic aldehydes have been shown to possess distinct physiological properties of their own [12]. For example, 5-HIAL has been shown to cause depression of firing rates of single neurons in the cerebellum and neocortex of rats [21], and DOPAL and the aldehyde derived from 3-methoxytyramine have been shown to inhibit rabbit platelet aggregation [22]. Furthermore, biogenic aldehydes have been reported to react with membranes and proteins [23, 24]. Since aldehydes are very reactive compounds, it has been suggested that the biogenic aldehydes could exert biological effects by interaction with, and modification of, tissue components (e.g. proteins or phospholipids) [25]. Elevated concentrations of biogenic aldehydes may also result in the formation of alkaloid condensation products, substances which may be involved in the development of alcohol dependence [3]. In the present study, the final recovery of the biogenic aldehydes, especially 5-HIAL, as the acid metabolites was decreased in incubations with acetaldehyde. Since a 100% final recovery was not obtained even in the absence of acetaldehyde, this suggests that the low recovery might be due to reactions of the biogenic aldehydes with tissue components. Furthermore, our results indicate that these reactions are potentiated in the presence of acetaldehyde. The decrease was more pronounced in incubations with erythrocytes, suggesting that the biogenic aldehydes did react with some specific component, e.g. hemoglobin. Acetaldehyde is known to react with and cause modification of hemoglobin [26], and recently it has been

proposed that antibodies against acetaldehyde-containing epitopes in acetaldehyde–protein adducts may be used in the identification of alcohol abuse [27].

In conclusion, our results show that both disulfiram and acetaldehyde interfere *in vitro* with the biogenic aldehyde metabolism in human erythrocytes, leukocytes as well as in platelets. It seems doubtful, however, that the metabolism of biogenic aldehydes would be markedly affected at the low levels of acetaldehyde (<10 μM) normally present in blood during ethanol metabolism *in vivo* [28]. It can be calculated, by applying Michaelis–Menten kinetics for a pure competitive inhibitor, that the oxidation of DOPAL would be inhibited by approximately 10% if DOPAL and acetaldehyde are present at concentrations 10 times below their K_m values (0.03 and 6 μM, respectively) [16]. However, much higher acetaldehyde levels (10–20-fold higher) are present during ethanol metabolism in combination with ALDH inhibition [29], and in such situations the inhibition would be more pronounced.

Acknowledgements—This work was supported by grants from the Swedish Medical Research Council (Grant No. 07526) and from the M. Bergwall, C. Groschinsky, L. Hierta and Hierta-Retzius foundations.

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